

Non-invasive Detection of Cell Death in MCF-7 Breast Cancer Cells by Hyperpolarized ^{13}C MRS

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Objective: Tumor response to therapy is assessed primarily in the clinic by monitoring reductions in tumor size. However, this approach lacks sensitivity since in many cases several weeks may elapse before there is evidence of tumor shrinkage. There is therefore a pressing need to develop non-invasive imaging techniques for monitoring tumor treatment response in the clinic. It has been shown recently that the uptake and metabolism of hyperpolarized $[1-^{13}\text{C}]$ pyruvate can be used to detect response to therapy using ^{13}C magnetic resonance spectroscopy (MRS) and spectroscopic imaging in murine models of lymphoma and prostate cancer [1,2]. The aim of the present study was to determine whether hyperpolarized ^{13}C MRS could be used to detect response to therapy in another, more drug resistant tumor cell line.

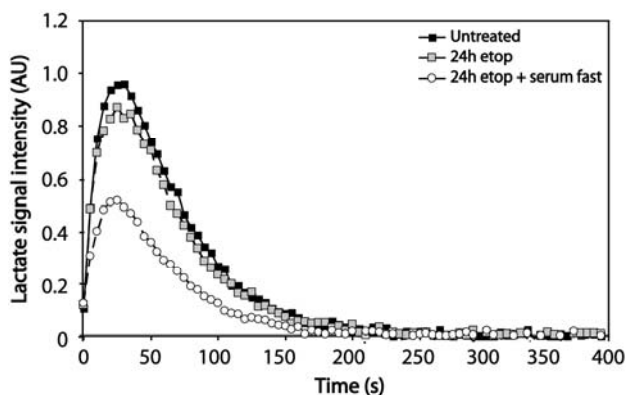


Fig 1. Effect of etoposide treatment on hyperpolarized pyruvate – lactate exchange in MCF-7 cells. ^{13}C signal intensity from $[1-^{13}\text{C}]$ lactate was measured as a function of time following addition of hyperpolarized $[1-^{13}\text{C}]$ pyruvate to a suspension of MCF-7 cells treated with etoposide for 24 h with and without serum fasting.

^{13}C pyruvate to a suspension of untreated MCF-7 cells resulted in detectable exchange of label with added lactate, in the reaction catalyzed by lactate dehydrogenase (LDH). There was no detectable cell death, nor change in LDH-catalyzed flux between pyruvate and lactate following treatment of cells with the DNA-damaging agent, etoposide, in comparison to untreated control cells. Serum starvation of cells 18h prior to the addition of etoposide however resulted in ~60% apoptosis and a 50% decrease in pyruvate – lactate exchange (Fig. 1). Treatment of cells with the protein kinase inhibitor, staurosporine (STS) for 4h resulted in 80-90% apoptosis as shown by fluorescent microscopy (Fig. 2a). Prior to this time point, minimal levels of cell death were detected. By 16h the majority of the cells were necrotic. A 60% drop in pyruvate - lactate exchange was observed after 4 h of STS treatment in comparison to untreated cells (Fig. 2b). Induction of low levels of apoptosis by 2 h resulted in a small decrease in pyruvate - lactate exchange. The largest decrease in exchange was evident at 16 h when the majority of cells were necrotic. These data show that the decrease in lactate - pyruvate exchange coincides with the induction of cell death in breast cancer cells, demonstrating that the polarized pyruvate experiment may be useful in differentiating between responders and non-responders following therapy in another tumor type.

Methods: MCF-7 human breast cancer cells were treated with either 100 μM etoposide (etop) or 1 μM staurosporine (STS) to induce cell death. The effect of serum starvation (1% fetal calf serum for 42 h), with and without etoposide, was also evaluated. Following treatment, hyperpolarized $[1-^{13}\text{C}]$ pyruvate - $[1-^{13}\text{C}]$ lactate exchange was assessed over a range of different treatment times: 75 mM hyperpolarized $[1-^{13}\text{C}]$ pyruvate [1] and 75 mM unlabeled lactate were added to a suspension of 2.6×10^7 MCF-7 cells in a 10 mm NMR tube, maintained at 37°C . ^{13}C spectra were acquired at 1 s intervals for 400 s using a low flip angle pulse in a 9.4 T vertical wide-bore magnet (Oxford Instruments). $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate peak intensities were adjusted for both polarization levels and cell number. Apoptosis and necrosis were assessed by microscopy using Hoechst 333258, YO-PRO-1 and propidium iodide (PI) stains. Hoechst 333258 is a UV-excitable, cell permeable dye that enables the quantitation of the total number of cells. The impermeant DNA-binding dye, YO-PRO-1, stains the nucleus of apoptotic and necrotic cells, whereas PI identifies the necrotic population only.

Results and Discussion: Addition of 75 mM hyperpolarized $[1-^{13}\text{C}]$ pyruvate to a suspension of untreated MCF-7 cells resulted in detectable exchange of label with added lactate, in the reaction catalyzed by lactate dehydrogenase (LDH). There was no detectable cell death, nor change in LDH-catalyzed flux between pyruvate and lactate following treatment of cells with the DNA-damaging agent, etoposide, in comparison to untreated control cells. Serum starvation of cells 18h prior to the addition of etoposide however resulted in ~60% apoptosis and a 50% decrease in pyruvate – lactate exchange (Fig. 1). Treatment of cells with the protein kinase inhibitor, staurosporine (STS) for 4h resulted in 80-90% apoptosis as shown by fluorescent microscopy (Fig. 2a). Prior to this time point, minimal levels of cell death were detected. By 16h the majority of the cells were necrotic. A 60% drop in pyruvate - lactate exchange was observed after 4 h of STS treatment in comparison to untreated cells (Fig. 2b). Induction of low levels of apoptosis by 2 h resulted in a small decrease in pyruvate - lactate exchange. The largest decrease in exchange was evident at 16 h when the majority of cells were necrotic. These data show that the decrease in lactate - pyruvate exchange coincides with the induction of cell death in breast cancer cells, demonstrating that the polarized pyruvate experiment may be useful in differentiating between responders and non-responders following therapy in another tumor type.

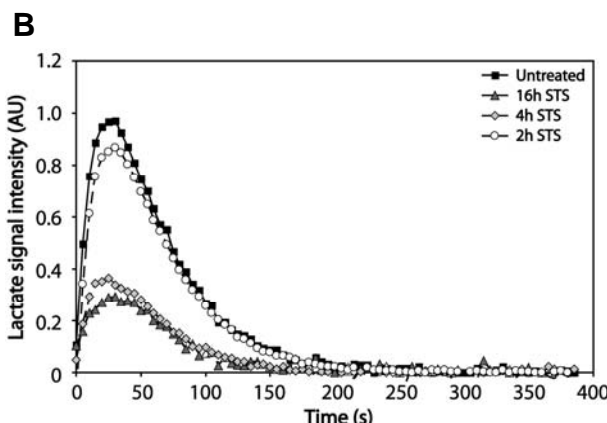
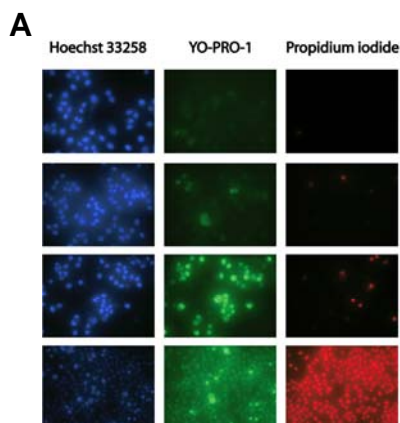


Fig 2. Detection of cell death in MCF-7 cells following STS treatment.

(A) Levels of apoptosis and necrosis detected by microscopy using YO-PRO-1 and PI stains respectively.

(B) Measurements of pyruvate – lactate exchange following STS treatment. ^{13}C signal intensity from $[1-^{13}\text{C}]$ lactate was measured following addition of 75mM hyperpolarized $[1-^{13}\text{C}]$ pyruvate to a suspension of STS-treated MCF-7 cells.

[1] Day SE et al. (2007) Nature Medicine. 13, pp.1382-1387

[2] Chen AP et al. (2008) Proc. Intl. Soc. Mag. Reson. Med. 16, pp.888